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MINI-REVIEW

Millimeter waves or extremely high frequency electromagnetic fields in the environment: what are their effects on bacteria?

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Abstract Millimeter waves (MMW) or electromagnetic fields of extremely high frequencies at low intensity is a new environmental factor, the level of which is increased as technology advance. It is of interest that bacteria and other cells might communicate with each other by electromagnetic field of sub-extremely high frequency range. These MMW affected *Escherichia coli* and many other bacteria, mainly depressing their growth and changing properties and activity. These effects were non-thermal and depended on different factors. The significant cellular targets for MMW effects could be water, cell plasma membrane, and genome. The model for the MMW interaction with bacteria is suggested; a role of the membrane-associated proton F_OF_1-ATPase, key enzyme of bioenergetic relevance, is proposed. The consequences of MMW interaction with bacteria are the changes in their sensitivity to different biologically active chemicals, including antibiotics. Novel data on MMW effects on bacteria and their sensitivity to different antibiotics are presented and discussed; the combined action of MMW and antibiotics resulted with more strong effects. These effects are of significance for understanding changed metabolic pathways and distinguish role of bacteria in environment; they might be leading to antibiotic resistance in bacteria. The effects might have applications in the development of technique, therapeutic practices, and food protection technology.

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Introduction

Electromagnetic field (EMF) has a significant interest for a long time due to effects of electromagnetic processes taking place in the environment on the functioning of living organisms and electromagnetic interconnections between them (Presman 1970). However, electromagnetic fields are of wide spectrum and different characteristics, and, on the other side, living organisms have a great diversity including bacteria and may have different sensitivity to EMF. A great number of data have been obtained in different labs for the last years and the clear evaluation of phenomenon, mechanisms, and consequences for application are required to be reviewed. It is especially of interest that low-intensity EMF has biological effects. The present review is focused on millimeter waves (MMW), some features and their effects on different bacteria due to the following reasons.

MMW (the wave length of 0.1 to 10 mm) or EMF of extremely high frequencies (of 30 to 300 GHz; with 1 GHz = 10^9 oscillations per second) has been discovered by Jagadish Bose and is relatively new and a widespread factor in the environment, the level of which is increased as technology advance. Nowadays, MMW of low-energetic and non-thermal intensity is used in satellite telecommunication, radiometry, traffic and military radars, and/or remote sensing devices. But the question is whether these devices are potentially harmful to living organisms, including bacteria (Pakhomov et al. 1998; Pakhomov and Murphy 2000; Silva 2001; Belyaev 2005; Bingi 2011; Gherardini et al. 2014; Redlarski et al. 2015). It was established that these devices produce MMW of much higher degree of coherence than do the sources of natural origin. This feature facilitates MMW discernment by bacteria and other cells (Hyland 2008; Redlarski et al. 2015). As a consequence, inhibitory effects on cells growth and their activity have been obtained with different bacteria (Torgomyan and Trchounian 2013). Regarding this feature, MMW are effectively used for treatment of hundreds of human disorders of the body surface or skin, mainly depressing pathogenic bacteria; they are applied for soil treatment and agricultural wastewater disinfection, in the food industry as protection technologies to inactivate pathogenic microorganisms at moderately low temperatures maintaining the nutritional quality of food and its safety, and in various biotechnologies (Rojavin and Ziskin 1998; Betskii et al. 2000; Banik et al. 2003; Usichenko et al. 2006; Geveke and Brunkhorst Ch 2007; Geveke et al. 2009; Ratushnyak et al. 2008; Shamis et al. 2008; Ukuku et al. 2008; Zand et al. 2010; Torgomyan and Trchounian 2013; Rodrigez-Chueca et al. 2014; Anton et al. 2015; Zohre et al. 2015).

It is of quite interest that according to the recent findings, bacteria and the other cells might communicate with each other through EMF possessing the ultrasonic irradiation by remissing secondary photons of sub-extremely high frequency range or MMW (Nikolaev 2000; Trushin 2003; Belyaev 2005; Cifra et al. 2011; Reguera 2011; Anton et al. 2015; Kucera et al. 2015). This might be affected by external MMW from different devices.

Therefore, MMW can imitate cellular control signals and induce bacteria to vibrate at specific frequencies. This change can alter membrane properties and change the metabolic processes in bacteria, particularly their responses to biologically active chemicals or antibiotics (Bulgakova et al. 1996; Caubet et al. 2004; Tadevosyan et al. 2008; Torgomyan et al. 2011b, 2012; Torgomyan and Trchounian 2012, 2013; Ahmed et al. 2015a).

The other interest is with that bacteria especially *Escherichia coli* is considered as a model organism with well-identified and documented structure, metabolic pathways, and heredity (Schaechter et al. 2001), and the effects of MMW on these bacteria and their mechanisms might give a significant basis to understand the EMF impact on other organisms including animal and human ones and to evaluate consequences for its applications.

In spite of several recent reviews on this topic (Shamis et al. 2012; Mishra et al. 2013; Torgomyan and Trchounian 2013; Gherardini et al. 2014; Yadollahpour et al. 2014; Redlarski et al. 2015), based on the arguments above and novel data about low-intensity MMW features and their bacterial effects for the last years, further clarifications are necessary for the conditions of MMW effects on bacteria, establishing main cellular targets especially water molecules and membrane-associated proteins like the proton F_OF_1-ATPase, understanding the mechanisms of affection, proposing directions of technique development, and offering further applications.

Noise and coherent MMW and studied bacteria

MMW or EMF of extremely high frequencies of low intensity (the power flux density of <0.06 mW/cm^2) and different types have been mainly used in the various study groups: (1) "noise" EMF (of broadband frequencies and accidentally changing phases) of 30 to 60 GHz (Ratushnyak et al. 2008) or 53 to 68 GHz (Trchounian et al. 2001; Isakhanyan and Trchounian 2005), and (2) coherent EMF (in time) usually within the range of 42 to 99 GHz (Bulgakova et al. 1996; Tadevosyan et al. 2007, 2008; Tadevosyan and Trchounian 2009; Cohen et al. 2010; Torgomyan and Trchounian 2011, 2012, 2015; Trchounian et al. 2012) frequencies (Table 1), using the respective generators (Russian made or others) and controlling frequency and power flux density. As critical comment, MMW of low intensities (<10 mW/cm^2) should be considered as non-thermal radiation with small increase of temperature (<1.5 °C) (Bulgakova et al. 1996) or its less increase (Torgomyan and Trchounian 2015). In spite of controversy with non-thermal effects of EMF of lower frequency reviewed (Bingi 2011; Shamis et al. 2012), temperature control was important to conclude about non-thermal mechanisms.

Furthermore, MMW at low intensity has been applied to a number of bacteria, and interesting effects have been observed with bacteria. The effects were established when a change can be clearly measured in a biological system after introduction to MMW stimuli. The findings obtained are in accordance with bioeffects discovered with weak physical and chemical stimuli (Burlakova et al. 2004). Among bacteria used were *E. coli* (Trchounian et al. 2001; Tadevosyan et al. 2007, 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011a, b, 2013a; Torgomyan and Trchounian 2011, 2012), *Enterococcus hirae* (Ohanyan et al. 2008, 2015; Torgomyan et al. 2012), *Lactbacillus acidophilus* (Soghomonyan and Trchounian 2013; Soghomonyan et al. 2014), *Staphylococcus aureus* (Bulgakova et al. 1996), and *Azotobacter* sp. (Ratushnyak et al. 2008) (see Table 1) as well as to *Bacillus firmus*, *Bacillus mucilaginous*, *Methanosarcina barkeri*, and others (Banik et al. 2003; Belyaev 2005; Zand et al. 2010; Gabrielyan et al. 2015).

The coherent MMW had more interest, as mentioned above (Hyland 2008). The clear effects of EMF at 51.8 or 53 GHz on *E. coli* have been shown well paying attention to the irradiation exposure and other conditions (Trchounian et al. 2001; Belyaev 2005; Tadevosyan 2007, 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011a; b, 2013a;

Table 1 MMW characteristics and their effects at low intensity on different bacteria

MMW characteristics	Extremely high frequency, GHz	Bacteria, species[a]	Effects	References
Noise	53.5–68	*Escherichia coli*	Inhibitory effect on cell growth after irradiation in distilled water; changes in membrane properties were observed	(Trchounian et al. 2001; Isakhanyan and Trchounian 2005)
			Stimulatory effect on cell growth after irradiation in buffered salt medium	(Isakhanyan and Trchounian 2005)
	30–60	*Azotobacter* sp.	Stimulatory effect on cells' number by small exposition period	(Ratushnyak et al. 2008)
Coherent	42, 54, 66, and 78	*Staphylococcus aureus*	Inhibitory and stimulatory effects on cell growth; changes in sensitivity to antibiotics	(Bulgakova et al. 1996)
	51.8 and 53	*Escherichia coli*	Inhibitory effect on cell growth depended on pH and enhanced in combination with antibiotics	(Tadevosyan et al. 2007, 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011b, 2012, 2013a)
		Enterococcus hirae	Inhibitory effect on cell growth, non-depended on pH and enhanced in combination with antibiotics	(Ohanyan et al. 2008, 2015; Torgomyan et al. 2012)
		Lactobacillus acidophilus	Inhibitory effect on cell growth enhanced in combination with antibiotics	(Soghomonyan and Trchounian 2013)
		Rhodobacter sphaeroides	Stimulatory or inhibitory effects on cell growth depended on the exposure period	(Gabrielyan et al. 2015)
	70.6 and 73	*Escherichia coli*	Inhibitory effect on cell growth and survival creating the conditions for facilitated effects of antibiotics	(Torgomyan et al. 2011b, 2013a; Torgomyan and Trchounian 2012)
	99		Inhibitory effect on cell viability after long exposure for irradiation; metabolic activity was not changed	(Cohen et al. 2010)

[a] Wild types

Torgomyan and Trchounian 2012, 2015; Zohre et al. 2015). This occurred within tenths of minutes following MMW; the optimal exposure time resulting in clear and reproducible data was 1 h. In contrast, the other EMF frequencies such as of 41 to 43 GHz; 61 and 99 GHz (Bulgakova et al. 1996; Cohen et al. 2010) probably had little effects on *E. coli*. On the other hand, the MMW effect can be enhanced with longer exposure period. Therefore, as it was shown for 99 GHz, the MMW effect was accumulated in the cell and revealed at higher exposure (Cohen et al. 2010) (see Table 1). However, repeated irradiation of bacteria by MMW (with interruption of 2 h) lowered the effects (Isakhanyan and Trchounian 2005).

Besides, the stimulatory effect of EMF of 2.5 GHz frequency was reported with *Salmonella typhimurium* cells number a long time ago (Hamnerius et al. 1985). This suggested different effects on bacteria for MMW. Indeed, the MMW stimulatory effect was determined with *E. coli* in vitro when buffered salts or hyperosmotic medium was used (Isakhanyan and Trchounian 2005). In addition, very low intensity ($<10^{-7}$ mW/cm^2) MMW have been also used in the study with soil nitrogen-fixing bacteria (Ratushnyak et al. 2008). "Noise" EMF of 30–60 GHz was applied for 5–15 min exposure to obtain effects on *Azotobacter* sp.: the significant increase of the number of these bacteria has been observed (Ratushnyak et al. 2008). These might be because of either direct or indirect activation of repair systems if any. Similar effects have been reported with *Rhodobacter sphaeroides* when 15-min irradiation by low-intensity coherent MMW of 51.8 GHz has stimulatory effect of bacterial growth but the prolongation of exposure up to 1 h caused the delay of the lag-growth phase and the decrease in their growth rate ~1.3-fold (Gabrielyan et al. 2015) (see Table 1).

Factors and conditions affecting MMW effects on different bacteria

Worldwide research groups have shown that MMW caused various and sometimes opposite effects on different bacteria: different factors and conditions become important. EMF wavelength or frequency, intensity, coherence, exposure duration, post-exposure time, and mediated and repeated irradiation were among those factors and conditions (Belyaev et al. 1993; Belyaev et al. 1996; Bulgakova et al. 1996; Scheglov et al. 2002; Alipov et al. 2003; Isakhanyan and Trchounian 2005; Tadevosyan et al. 2007, 2008; Torgomyan et al. 2011a, 2012; Zohre et al. 2015). In addition, bacterial growth logarithmic (exponential) or stationary phase, anaerobic or aerobic conditions of cultivation, composition of nutrient media, cell number or density, genetic features, peculiarities of metabolism, and membrane structures and properties in bacterial species or strains and others should be taken into consideration when describing MMW effects on bacteria (Torgomyan and Trchounian 2013).

Depressive effects of EMF of different frequencies on the growth and viability of *E. coli* have been installed in our lab (Tadevosyan et al. 2007, 2008; Torgomyan et al. 2011a; b; Torgomyan and Trchounian 2011, 2012, 2013) and by the other group (Cohen et al. 2010). The direct irradiation of bacteria on solid nutrient medium with MMW reduced the numbers of colonies compared to the established non-irradiated control (Fig. 1). As it was shown by Torgomyan et al. (2013a), the inhibitory effects on the growth were more under direct irradiation of cells on solid medium than their irradiation in aqueous suspension The effects were more with 51.8 and 53 GHz (see Fig. 1). Interestingly, MMW affected not only the reduction in the colonies numbers, but also their morphology and sizes (Torgomyan et al. 2011a).

The effects of low-intensity MMW have some similarities and differences with a number of bacteria studied (see Table 1). In addition to *E. coli*, *E. hirae* and *L. acidophilus* were sensitive to MMW but in a lesser degree: inhibitory effects have been shown depending on frequency and exposure (Ohanyan et al. 2008, 2015; Soghomonyan and Trchounian 2013). These effects were observed with bacterial growth in liquid media and on solid nutrient one as well as with their survival in minimal salt medium. These data are similar to those reporting about difference between Gram-negative and Gram-positive bacteria by radiofrequency irradiation near telecommunication stations when Gram-positive bacteria like *Bacillus* and *Clostridium* spp. or *Corinebacterium striatum* were less sensitive to EMF and can survive (Adebayo et al. 2014).

In order to reveal additional mechanisms, different bacterial strains were photo documented using scanning electron microscope and X-ray micro-analyzed. Based on results, irradiated with 53 GHz EMF for 1 h, bacterial cells of *E. coli* have been discovered to increase their volumes on ~60 %, while *E. hirae* cells swelled ~14 % (Torgomyan and Trchounian 2013; Ohanyan et al. 2015). These effects were interesting since morphology changes may provide explanation to the bactericidal effects of MMW and changing sensitivity of bacteria to chemicals and antibiotics (hereafter). The difference may be due to membrane dissimilarities, as long as *E. coli* is Gram-negative, while *E. hirae* is Gram-positive bacteria. This has been employed to explain different behavior of these bacteria induced by MMW (Rodrigez-Chueca et al. 2014).

MMW effects on bacteria might be considered as specific ones since different effects have been obtained with EMF of other frequencies (Ahmed et al. 2015b; Hwang et al. 2015; Redlarski et al. 2015).

Interestingly, inhibitory effects of MMW have been observed by Ahmed et al. (2015a) with some pathogenic molds:

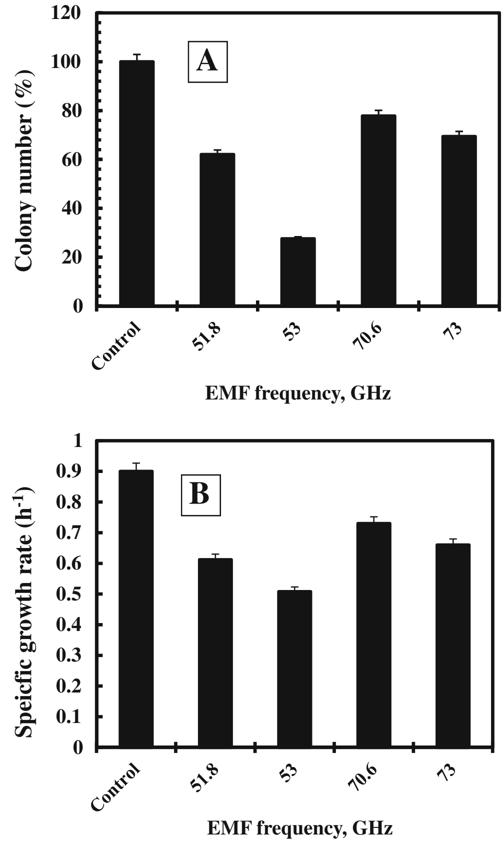

Fig. 1 The changes in *E. coli* K12(λ) colony-forming units number spread on solid nutrient medium (**a**) and specific growth rate (**b**) in liquid medium transferred from bi-distilled water after irradiation by low-intensity MMW. Bacteria were irradiated by EMF at the frequencies of 51.8, 53, 70.6, and 73 GHz; irradiation time was 1 h; the power flux density was of 0.06 mW/cm^2. In the control, bacteria were non-irradiated (100 %, **a**). The data were adapted from Tadevosyan et al. 2008; Torgomyan et al. 2011a, 2013b; and Torgomyan and Trchounian 2012, 2015

increasing of the exposure to 1 h resulted to complete inhibition of their growth.

Role of water in MMW effects

Low-intensity MMW inhibitory effects have been obtained with *E. coli* cells in aqueous suspension and on solid surfaces but the effects were different (see Fig. 1). Furthermore, the strong effects in aqueous suspension were at the frequencies (50.3, 51.8, 64.5, 65.5, 95, 105 GHz), which were resonant for water molecules (Fesenko et al. 1995; Betskii et al. 2000; Sinitsyn et al. 2000; Tadevosyan et al. 2007). Therefore, MMW effects are suggested, might be mediated by water. But, on the other hand, EMF of 41 to 43 GHz, 53 GHz, 70.6 GHz, and 73 GHz frequencies were also absorbed by *E. coli* and had resonant-like bactericidal effects (Guofen et al. 2002; Torgomyan et al. 2011a). It was shown that *E. coli* growth after irradiation of bacterial suspension in bi-distilled water was more pronounced with 53 and 51.8 GHz than with 73 and 70.6 GHz (see Fig. 1). The effects are not only linked with the energy of MMW, because the higher is the frequency of EMF the greater is the energy of a photon associated with it. Differences also are attached with the different MMW effects on the structure of water molecules in the surrounding medium. This may be the primary mechanism to create the conditions for biological response to MMW.

The mediated effects of 70.6 and 73 GHz by irradiated medium used (bi-distilled water, assay buffer, or growth medium) on *E. coli* growth were different from those for 51.8 and 53 GHz (Tadevosyan et al. 2007) and non-marked (Torgomyan et al. 2011a). It was found that with 70.6 and 73 GHz, the cell growth was similarly suppressed after direct irradiation of *E. coli* in liquid or on solid medium. Interestingly, for 51.8 and 53 GHz, the growth decrease after *E. coli* aqueous suspension irradiation was smaller than the direct irradiation of bacteria on solid medium. It could be suggested that MMW impinging with water is likely to lose its energy, but the transferred amount of energy is dependent on a specific frequency (Fesenko et al. 1995; Golovleva et al. 1997; Sinitsyn et al. 2000; Torgomyan et al. 2011a; Torgomyan and Trchounian 2012).

Thus, water, which is a major constituent of liquid surrounding of bacteria and different biological systems as well, could be a target for MMW. The latter at low energy might change water cluster structuring and features leading to increased chemical activity or hydration of cellular structures and components, as membrane-associated proteins. Indeed, the changes of water absorbance, electroconductivity, and pH by low-intensity EMF at different frequencies have been shown (Tadevosyan et al. 2007; Torgomyan et al. 2011a). It has been established that these changes of water features might be specific depending on EMF frequencies and long term (Fesenko et al. 1995; Golovleva et al. 1997; Sinitsyn et al. 2000; Belyaev 2005; Novoselova et al. 2005; Tadevosyan et al. 2007; Bingi 2011; Torgomyan et al. 2011a). Importantly, more visible evidences have been brought up with the increase of optical density of bi-distilled water in the near-ultraviolet region (200–340 nm) exposed to 51.8 and 70.6 GHz during 1 h. For pH, in case of 51.8 GHz, the slight acidification of bi-distilled water occurred, but in cases of 70.6 and 73 GHz water pH adjusted to 6.0 was increased, and water pH at 8.0 was decreased (Tadevosyan et al. 2007; Torgomyan et al. 2011a, 2013b). These effects might be explained by that MMW energy could be accumulated into the water structures triggering oscillations in them and increasing H^+ and OH^- dispersions (Fesenko et al. 1995; Sinitsyn et al. 2000; Guofen et al. 2002; Belyaev 2005; Novoselova et al. 2005; Cohen et al. 2010).

Moreover, formation of reactive forms of oxygen during irradiation of aqueous solutions with EMF of 41 GHz has been determined (Potselueva et al. 1998) that could provide additional mechanism for MMW effects on cells.

Cellular mechanisms of MMW effects in bacteria, model for MMW interaction with bacteria, and role of the F_OF_1-ATPase

In addition to water, the cellular mechanisms of MMW effects in bacteria might involve different structures and components like bacterial plasma membrane with its surface characteristics, substances transport, and energy-transforming processes (Neshev and Kirilova 1994; Bulgakova et al. 1996; Kandashev and Savin 1997; Trchounian et al. 2001; Tadevosyan et al. 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011a; Torgomyan and Trchounian 2012, 2013) and genome (Belyaev et al. 1993; Belyaev et al. 1996; Scheglov et al. 2002; Alipov et al. 2003; Ruediger 2009) (Fig. 2). In should be noted that low-intensity (<10 mW/cm^2) MMW do not cause direct physical damage, ionization, or heating (<1.5 °C) of exposed surface bacteria in thin layer (Betskii et al. 2000; Belyaev 2005; Hyland 2008), and these MMW energy is not sufficient to destabilize hydrogen bonds of cellular macromolecules as proteins and nucleic acids or break the chemical bonds in DNA molecules (Ruediger 2009).

To explicate the mechanisms of MMW interaction with bacteria resulting in inhibitory or stimulatory effects, quantum-mechanical approach and physics of non-equilibrium and nonlinear systems are applied and the model is proposed (Sinitsyn et al. 2000). Under MMW irradiation, certain cellular structures or components can be excited by interaction with coherent excitation of a specific frequency and precise value which depends on particular electric dipoles characterizing active material (Betskii et al. 2000; Belyaev 2005; Bingi 2011); Bose phonons condensed in a biological system can be formed (Anton et al. 2015). The MMW wavelength is largely compared with the spatial dimension of the system, which coherent excitation requires energy from irradiation. The system becomes strong excited triggering basic biological processes, and then the system acts to stabilize this mode and induces deformations (Hyland 2008).

The changes in water features by their turn can alter membrane proteins conformation, their hydration degree, and other properties resulting in their altered activity (Neshev and Kirilova 1994; Kandashev and Savin 1997; Trchounian et al. 2001; Belyaev 2005; Tadevosyan et al. 2008; Ukuku et al. 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011b; Torgomyan and Trchounian 2013; Shamis et al. 2012; Torgomyan and Trchounian 2011, 2012). All this can even change cells' morphology and sizes (Torgomyan et al. 2013a). In addition, difference between the effects of EMF of different frequencies on bacteria might also be associated with their primary resonant interaction with cells. MMW, therefore,

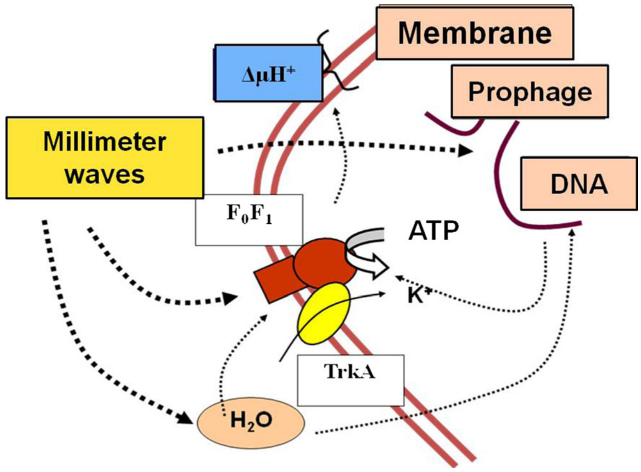

Fig. 2 Probable ways and three main primary cellular targets for MMW in bacteria—membrane proteins (the proton F_0F_1-ATPase (F_0F_1), K^+ transporter (TrkA in *E. coli*), water molecules (H_2O), and genome. F_0F_1 generates proton electrochemical gradients across the membrane ($\Delta\mu_{H^+}$). For the others, see the text

can cause bacteria to vibrate and alter proteins conformations and activity. The model presented is of interest and should be developed but it is not accepted by different research groups yet (Bingi 2011; Cifra et al. 2011; Kucera et al. 2015; Redlarski et al. 2015; Buchachenko 2016).

It was revealed in our lab (Trchounian et al. 2001; Tadevosyan et al. 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2011b, 2012; Torgomyan and Trchounian 2011, 2012; Soghomonyan and Trchounian 2013; Gabrielyan et al. 2015; Ohanyan et al. 2015) that EMF of 51.8, 53, 70.6, and 73 GHz frequencies altered ion (H^+ and K^+) transport processes and enzymatic activities of plasma membranes in bacteria. In particular, the changes in the activity of proton F_OF_1-ATPase, key membrane-associated enzyme of bioenergetic relevance, responsible for generation of proton electrochemical gradients across the membrane (Trchounian and Sawers 2014), were determined with MMW irradiation of bacteria (Torgomyan et al. 2012; Soghomonyan and Trchounian 2013; Soghomonyan et al. 2014). This ATPase can interact with the K^+ transport (TrkA in *E. coli*) system and other membrane proteins having additional functions in bacteria (Trchounian 2004). The obtained results point out that the F_OF_1-ATPase can be among the primary cellular targets for MMW (see Fig. 2). The effects were similar with non-thermal specific influence of EMF of lower frequency on some bacterial and archaeal enzymes (Shamis et al. 2012). In addition, the changed bacterial sensitivity to chemicals, especially to inhibitors of membrane proteins and antibiotics with the membranotropic action (see hereafter), indicates the main role of membrane in the cellular responses to MMW. The appropriate changes in the membrane properties and activity mentioned have been clearly determined. This has been also suggested with molds (Ahmed et al. 2015a). Interestingly, a role of ATPase might be common for different cells: increased synthesis of ATP in biological effects of EMF is also offered for human cells (Buchachenko 2016).

Then, the oscillations in the regulatory DNA sequences and conformational changes in bacterial genome are likely as well (Belyaev 2005). The effective rearrangement of electron subsystem levels in DNA molecule might be induced by EMF of 41.3 and 51.8 GHz: the effects were observed at very low intensity (~10^{-16} mW/cm^2) (Belyaev et al. 1996; Ruediger 2009). There are many problems with determination methods, replicability, and explanation of the effects; additional study is required. Also, it is more likely that MMW genotoxic effects were mediated in other ways including membranous action and disturbance of DNA-repair processes (Ruediger 2009; Gherardini et al. 2014).

Moreover, the mechanism with MMW transformation into informative biological signals is also offered (Pakhomov et al. 1998; Betskii et al. 2000; Pakhomov and Murphy 2000; Guofen et al. 2002; Belyaev 2005; Anton et al. 2015). MMW could mimic control signals produced by cells driving a cellular response by correction of metabolic processes, especially in the plasma membranes. Nevertheless, it is obvious that bacterial cell response to MMW can be integrative of different mechanisms. But the updated experimental results are not enough to give a general imagination about MMW interaction with bacteria and other cells.

Changes in sensitivity of bacteria to antibiotics by MMW and their application

In recent years, it has been established by our group (Tadevosyan et al. 2008; Torgomyan et al. 2011a, b; Torgomyan and Trchounian 2012) that different antibiotics of broad-spectrum bacteriostatic or bactericide effects as tetracycline (polyketide), chloramphenicol, kanamycin (aminoglycoside), and ceftriaxone (β-lactam, third-generation cephalosporin) at their minimal inhibitory concentrations (MIC) affect *E. coli* growth and survival and these effects could be significantly enforced by low-intensity MMW (Table 2). Similar effects have been obtained with *E. hirae* (Torgomyan et al. 2012, 2013b; Ohanyan 2012; Ohanyan et al. 2015) and *L. acidophilus* (Soghomonyan and Trchounian 2013; Soghomonyan et al. 2014) for different antibiotics generally having membranotropic action (see Table 2). MMW is assumed to change the sensitivity to antibiotics or resistance to these chemicals; these changes might depend on EMF frequency. Various changes as membrane permeabilization (McMurry et al. 1986; James et al. 2009); dissipation of proton-motive force generated by the proton F_OF_1-ATPase under fermentation conditions; disturbances in H^+, K^+, and Na^+ transport; alterations in appropriate membrane transport systems; and lowering ATP level (Trchounian 2004;

Torgomyan and Trchounian 2013) might increase the sensitivity of bacteria to antibiotics (Fig. 3). In addition, alterations in DNA gyrase and other changes in gene expression or protein synthesis processes (Cambau and Gutmann 1993) and differences in membrane proteome (Xu et al. 2006) play an important role in changing the sensitivity to antibiotics.

It is of interest that these results seem to be in accordance with the data of changed sensitivity of *S. aureus* to various membranotropic antibiotics by MMW with non-thermal intensity (see Table 2). The inhibitory and stimulatory effects with *S. aureus* depended on exposure to MMW and concentrations of antibiotics (Bulgakova et al. 1996), but the results were not clear without determination of MIC. There were also data about MMW effects on the sensitivity of the other pathogenic bacteria to antibiotics (Rojavin and Ziskin 1998; Betskii et al. 2000). In addition, that is likely due to the bioelectric effect with radio frequency alternating electric current (10 MHz) for *E. coli* biofilms treated with the other antibiotics (Caubet et al. 2004). The sharp increase of the efficacy of the antibiotics in biofilms was explained due to a specific action of the radio frequency EMF on water molecules in an aqueous surrounding or in biofilms matrix. All these might be also explained by effects of extremely low concentrations of chemicals and weak EMF when nanosized particles composed of ordered water structures are formed and these particles associations are responsible for unique properties of highly diluted aqueous solutions of biologically active chemicals ($<10^{-6}$ mol L^{-1}) (Ryzhkina et al. 2012; Konovalov et al. 2015). Interestingly, the weak EMF is mandatory for formation of nanoparticles associations (Ryzhkina et al. 2012). But the role of membrane properties changes and membrane-associated proteins is not excluded.

Many antibiotics are known to cause modifications and disruptions of the plasma membrane in bacteria, those reflecting in changes of their properties, morphology, and biochemical processes (Nakae and Nakae 1982; Chung et al. 2006; Kohanski et al. 2007; Guliy et al. 2008). This might be occurred in combined action with MMW on the same membranous targets as well. As it has been shown in recent years, 51.8 and 53 GHz EMF in combination with antibiotics suppressed H^+ and K^+ transport, in which the main role was played by the proton F_OF_1–ATPase (Torgomyan et al. 2011b, 2012; Ohanyan et al. 2015). The latter can be a target for a variety of antibiotics which can bind to, change properties, and inhibit the proton F_OF_1-ATPase, as reviewed by Hong and Pedersen (2008). This in its turn can damage cell energy status. Thus, the enhanced cell death could be resulted (see Fig. 3) and detected. This explanation is probably that potential antibiotic combination targets in bacteria provide an adaptive stress response reinforcing the effects of antibiotics (Lee et al. 2009). Changes in the other cellular processes as protein synthesis and DNA replication should not be ruled out.

It is of quite interest that antibiotics irradiated with MMW have more strong inhibitory effects (1.2-fold for kanamycin and tetracycline) on *E. coli* than irradiation of bacteria in combination with non-irradiated antibiotics (Torgomyan and Trchounian 2015) (see Table 2). A similar result has been obtained with irradiated ceftazidime (Ohanyan et al. 2015) (see Table 2). For the application, a further study is required. As mentioned above, in the process of irradiation, MMW energy partially absorbed by the water is changing its molecular structure; the formation of nano associations of antibiotics is possible (Ryzhkina et al. 2012; Konovalov et al. 2015). Such water has an increased ability to transfer that energy to solved antibiotics resulting in their aggressive action. In this case, the structure of an antibiotic is important.

Improved bacterial biofilm control by EMF in combination with various antibiotics is a phenomenon established with a pulsed EMF (McLeod et al. 1999; Pickering et al. 2003). This EMF has been recently shown to increase antimicrobial activity against *Staphylococcus epidermitis* of the other substance, e.g., lime oil (Matewele 2010). This is likely due to the effect of metronidazole in low concentration on infusoria when MMW of 167.1 GHz was applied (Rogacheva et al. 2008).

Changing the sensitivity of bacteria to antibiotics by MMW irradiation can be important for the understanding of antibiotics resistance in the environment. In this respect, it is interesting that bacteria survived near telecommunication-based stations like *Bacillus* and *Clostridium* spp. have been found to be multidrug resistant (Adebayo et al. 2014). Furthermore, the changing antibiotic activity for various bacteria by low-intensity MMW can be viewed as a new phenomenon which may also have a concrete explanation and a wide application in therapeutic practice, agriculture, and the food industry. It would be effective to decrease using antibiotics by applying MMW at low intensity. This could also be a novel and important way to prevent the development of resistance to antibiotics. The other interesting application might be the development of a technique to generate MMW or to use for combined effects with antibiotics.

Concluding remarks

It can be declared that MMW even with low intensity becomes a novel environmental factor influencing a variety of bacteria and their populations. Perhaps, MMW has bactericidal and the other actions, leading to a change in characteristics and metabolic pathways in bacteria and to their antibiotic resistance. These effects and the interference of MMW in communications between cells would be a special point of interest in bacterial physiology and ecology.

A progress in understanding of MMW interaction mechanisms and their cellular targets in bacteria as water molecules and membrane-associated proteins especially the proton

Table 2 The changes of bacterial sensitivity to antibiotics by coherent MMW of low intensity

MW characteristics, extremely high frequency, GHz	Bacteria, species	Antibiotics, minimal inhibitory concentrations	Effects	References
42, 54, 66, and 78	*Staphylococcus aureus*	Gramicidin S; tyrocidin	Inhibitory and stimulatory effects for cell growth depended on exposure to irradiation and antibiotics concentrations; changes in sensitivity to antibiotics were observed	(Bulgakova et al. 1996)
51.8 and 53	*Escherichia coli*	Chloramphenicol, 4 μM; ceftriaxon, 0.4 μM; kanamycin, 15 μM; tetracycline, 4 μM	Enhanced inhibitory effects on cells growth after irradiation of bacteria	(Tadevosyan et al. 2008; Tadevosyan and Trchounian 2009; Torgomyan et al. 2012; Torgomyan and Trchounian 2012)
53		Chloramphenicol, 4 μM; ceftriaxon, 0.4 μM; kanamycin, 15 μM; tetracycline, 4 μM	Enhanced inhibitory effects on cells growth after irradiation of antibiotics	(Torgomyan and Trchounian 2015)
51.8 and 53	*Escherichia coli*	Ceftazidime, 20 μM	Enhanced inhibitory effects on cells' growth after irradiation of antibiotics	(Ohanyan et al. 2015
	Enterococcus hirae	Ceftriaxon, 100 μM; kanamycin, 200 μM	Enhanced inhibitory effects on cells' growth after irradiation of bacteria	(Torgomyan et al. 2012, 2013b; Ohanyan et al. 2015)
		Ampicillin, 1.4 mM; dalacin, 0.4 mM	Enhanced inhibitory effects on cells' growth and survival after irradiation of bacteria; 53 GHz was more stronger in the combination with dalacin	(Ohanyan 2012; Torgomyan et al. 2013a)
	Lactobacillus acidophilus	Ceftazidime, 16 μM	Enhanced inhibitory effects on cells' growth and survival after irradiation of bacteria	(Soghomonyan and Trchounian 2013)
70.6 and 73	*Escherichia coli*	Chloramphenicol, 4 μM; ceftriaxon, 0.4 μM; kanamycin, 15 μM; tetracycline, 4 μM	Enhanced inhibitory effects on cells' growth and viability after irradiation of bacteria	(Torgomyan et al. 2011a, 2013a; Torgomyan and Trchounian 2012, 2015)

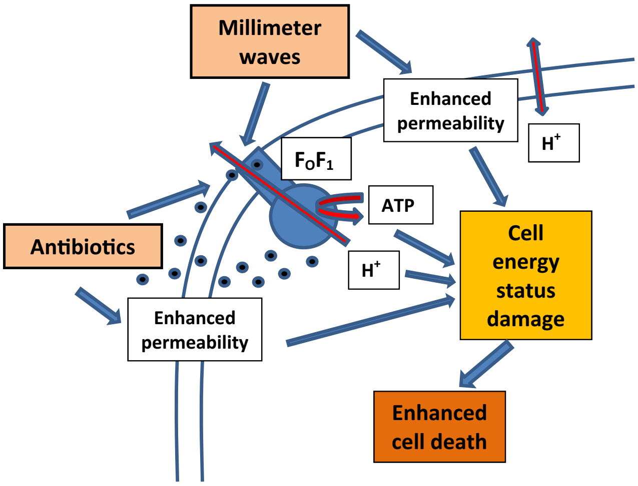

Fig. 3 Combined actions of MMW and antibiotics on bacteria. MMW signals are primarily transformed to change water cluster structuring, membrane proteins properties and activity, and other cellular structures. Both MMW and antibiotics affect the proton F_0F_1-ATPase. These all create conditions when the action of antibiotics is facilitated to bring enhanced cell damage especially changing cell energy status and subsequently causing cell death. For the others, see the text. The based results were from Torgomyan et al. 2011b and Torgomyan and Trchounian 2013, 2015

F_OF_1-ATPase can be stated. This knowledge will serve as a basis for extrapolating the results obtained with bacteria to future microbial and other biotechnologies, to animal and human issues. It matters, MMW application, in the food industry to increase the safety and nutritional properties; in biotechnology—to obtain biomass and end-products; and in therapeutic practice—to treat the broad range of bacterial diseases. The results are promising in application to change the distribution of bacteria in the biosphere. Then, the important aspect also is to develop technique and to define MMW exposure conditions and duration depending on the specific goals.

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Compliance with ethical standards

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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